Synchrotron IR Spectromicroscopy of Living Cells

Spectra Show Changes Induced by Dioxin

A research team from Berkeley Lab has demonstrated that infrared spectromicroscopy with synchrotron light can be useful for examining dioxin-induced changes in individual living cells. Their work provides new clues about what happens to human cells exposed to dioxins. They used the Fourier-transform infrared (FTIR) microscope at Beamline 1.4.3 to study the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on liver cells.

The dioxin they studied is one of the most potent of an important class of toxins, the polychlorinated aromatic hydrocarbons. Such toxins can cause cancer, birth defects, and altered hormone levels, among other things. In the body, particularly in liver cells, TCDD binds to the aryl hydrocarbon receptor. This binding induces increased production of cytochrome P4501A1 by increasing expression of the CYP1A1 gene that manufactures it. Cytochrome P4501A1 is involved in metabolizing foreign compounds, such as aromatic hydrocarbons, in humans.

In the study, three experimental groups of human hepatocellular carcinoma (HepG2) cells (cells from a liver tumor) were exposed to TCDD at different concentrations for 20 hours, and HepG2 cells in a control group were kept in an incubator for 20 hours with no TCDD exposure. Spectra from cells in the experimental groups and in the control group were then obtained with the infrared microscope. In addition, some cells from each group were analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) to track the effect of TCDD on expression of the CYP1A1 gene. A correlation between spectroscopic and RT-PCR data would demonstrate that the spectroscopic changes were indeed related to the pathway of CYP1A1 expression.

Absorption spectra were obtained at wavenumbers between 4000 and 650 cm^{-1}. Significant absorption differences between the treated samples and the control samples were evident for wavelengths associated with stretching vibrations in two bond types, phosphate and C–H. For phosphate, the intensity for the symmetric stretch band increased relative to that for the asymmetric stretch band with increased TCDD concentration. This finding agrees with previous studies in cancer cells. One thing, however, was different about the phosphate bands in the present study. They did not shift in wavelength. Such a shift would have indicated increased or decreased hydrogen bonding with exposure to TCDD. (Hydrogen bonding is important for maintaining the three-dimensional structure of a protein.) The wavelengths at which the phosphate vibrations were found and the fact that they did not vary show that phosphate has weak hydrogen bonding in HepG2 cells and that this does not change with TCDD exposure. This behavior is different from that seen in the early response to oxidative stress. The band representing methylene (CH_2) stretch decreased and that for methyl (CH_3) stretch increased with increasing TCDD concentration. Thus, the number of methyl groups relative to the number of methylene groups increased with greater TCDD exposure. This may indicate increased DNA methylation, which some researchers have suggested may cause gene inactivation—one possible mechanism by which TCDD could take its toxic toll.

The RT-PCR results showed the expected increase in CYP1A1 gene expression with increasing exposure to TCDD. Comparing these results with the increases in intensity for the symmetric versus asymmetric phosphate bands gave excellent correlation ($r^2 = 0.96$). This strong correlation shows that the spectromicroscopy measurements succeeded in tracking real changes that are associated with induction of the CYP1A1 gene.

Using synchrotron radiation for FTIR spectromicroscopy has several advantages over other techniques. Spectromicroscopy with globar infrared sources is limited to resolutions around 75 microns. With the brightness of synchrotron radiation from the ALS, resolutions of 10 microns or less are obtainable. Thus, a single cell can easily be studied. Because infrared techniques do not damage a living sample or require extensive preparation, this approach may prove ideal for monitoring cellular exposure to environmental pollutants. ■

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H.-Y.N. Holman, R. Goth-Goldstein, M.C. Martin, M.L. Russell, and W. R. McKinney, "Low-dose responses to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in single living human cells measured by synchrotron infrared spectromicroscopy," *Env. Sci. & Technol.* **34**(12), 2513–2517.

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SYNCHROTRON IR SPECTROMICROSCOPY OF LIVING CELLS

Spectra Show Changes Induced by Dioxin

- **Living human cells exposed to dioxin in varying concentrations**
- **Single cells studied by IR spectromicroscopy and RT-PCR**
  - *Bright synchrotron light gives order of magnitude better resolution than globar*
- **Significant spectroscopic changes observed for PO_2 and C–H stretching vibrations**
  - *Weak hydrogen bonding in phosphate is not changed with dioxin exposure*
  - *Number of methyl groups increased relative to number of methylene groups*
- **Spectroscopic changes correlated well with RT-PCR data**
  - *Spectromicroscopy measured real changes that relate to dioxin action*
  - *Infrared spectromicroscopy shows promise as a diagnostic tool*

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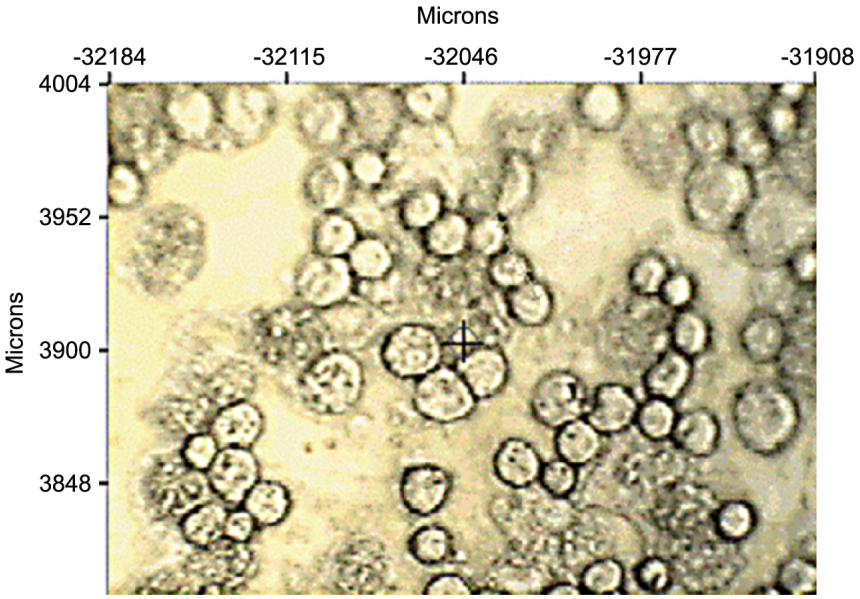
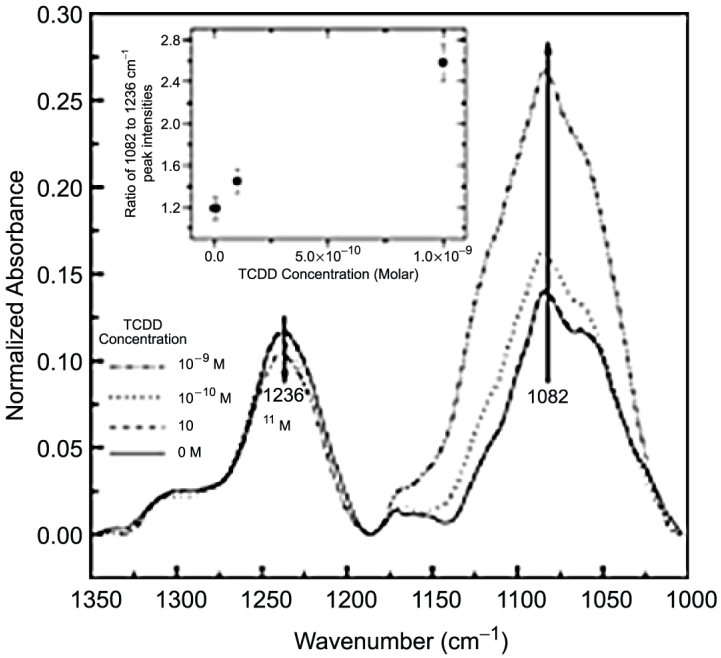
SYNCHROTRON IR SPECTROMICROSCOPY OF LIVING CELLS

Spectra Show Changes Induced by Dioxin



HepG2 cells as seen through the infrared microscope on Beamline 1.4.3. Spot sizes for spectromicroscopy were 10 μm or less, allowing study of individual cells.



Infrared spectra near phosphate bands at 1236 cm^{-1} (asymmetric stretch) and 1082 cm^{-1} (symmetric stretch) show differences for cells treated with varying concentrations of TCDD.

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